



Environmental and phenotypic heterogeneity of populations at the trailing range-edge of the habitat-forming macroalga *Fucus serratus*

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ABSTRACT

Empirical work on the dynamics of range limits of species distributions often lack replications of edge populations. We compared the local environment and performance of two groups of geographically peripheral populations of the foundation intertidal alga *Fucus serratus* L. at its southern range boundary in the NW Iberian Peninsula. Two populations were located on the Western Galician coast in large embayments or *rias*, and the other two on a Northern open coastal stretch in Lugo province. Sharp differences were detected in the local environment and performance of the two groups of populations. While recruitment was quite consistent throughout the year in *rias*, it was very limited and variable in Lugo. Furthermore, thalli from *rias* were severely damaged following their transplantation in Lugo, and poor conditions of local adult plants were detected there in subsequent years. These results suggest a higher vulnerability of Lugo populations under new climate conditions, while western *rias*, strongly influenced by upwelling events, feasibly act as contemporary refugia for this species. If sustained over time, these refugia may mitigate the retreat of the seaweed's rear-edge predicted by large spatial scale models.

1. Introduction

In the context of contemporary climate change, responses of geographically peripheral populations, especially those located at the low-latitude limit (rear edge) of species distributions (Hampe and Petit, 2005), are of crucial importance, as they largely determine the sensitivity of species to the shifting environment (Rehm et al., 2015). Edge populations are commonly smaller in size, more spatially isolated and genetically depauperate compared to core populations (Kawecki, 2008; Sexton et al., 2009). These peripheral populations, which often occur in 'marginal' habitats considering the ecological niche of the species, are prone to extinction due to perturbations (Kawecki, 2008). This is implicitly assumed by many model-based analyses which predict a climate-driven retreat of the trailing edge of species distributions (e.g. Lima et al., 2007; Sunday et al., 2012; Jueterbock et al., 2013).

However, geographically peripheral populations do not always occur in marginal, unfavourable settings. Sometimes they occupy present-day climate refugia, i.e. 'safe', favorable habitats in a regional environment more vulnerable to climate change (Ashcroft, 2010). This spatial heterogeneity in habitat quality, with the potential presence of climate refugia at the range margin rather than a simplistic gradient of less suitable habitat from core to periphery, has seldom been considered in climate change predictions (but see Lennon et al., 2002; Lourenço

et al., 2016).

Moreover, peripheral populations may be locally adapted or acclimated to low-quality, marginal conditions (Kawecki, 2008; Sexton et al., 2009; Rehm et al., 2015). In fact, the model of reduced genetic diversity at the periphery has been challenged. If edge populations maintain significant genetic diversity in their adaptive traits, they may diverge from central populations as a consequence of habitat differences and limited gene flow (Eckert et al., 2008). Reciprocal transplant experiments specifically test for local adaptation, which is manifest when local genotypes outperform those from other origins (Kawecki and Ebert, 2004). Plastic mechanisms can also adjust organisms to local conditions. Genetic and phenotypic responses to marginal habitats may facilitate the persistence of edge populations, mitigating the forecasted erosion of low-latitude range limits in climate change scenarios (Stillman, 2003; Sanford and Kelly, 2011).

Empirical work on the dynamics of range limits of species distributions often lack replications of edge populations (Sexton et al., 2009). Comparisons of core-edge populations are more common than edge-edge population comparisons, and though frequently overlooked, populations from the same range boundary may not constitute a homogeneous pool either in the environment they experienced or in their adaptive abilities. These kinds of comparisons are needed to achieve robust conclusions concerning the conservation value and

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vulnerability of edge populations to climate change.

In coastal ecosystems, foundation species such as canopy-forming macroalgae are increasingly threatened by climate change (Jueterbock et al., 2013). The changes induced in these species will trigger modifications in the whole coastal ecosystem, as large, canopy-forming seaweeds play a key role in rocky shore assemblages, providing shelter and food for a high diversity of organisms (Schiel, 2006). Studies have shown that the rear edge of several populations of these habitat-forming algae is retreating (e.g. Smale and Wernberg, 2013; Nicastro et al., 2013; Fernández, 2016). Due to the scarcity of data on the adaptive potential of edge populations, we are uncertain as to the extent of these changes and the applicability of model projections for future climate scenarios (Jueterbock et al., 2013, 2014; Bennett et al., 2015). Moreover, the identification of potential contemporary refugia at range boundaries, is also largely unexplored in these foundational species (but see Lourenço et al., 2016).

The Iberian Peninsula has been a climatic refugium during the Last Glacial Maximum for several species of North Atlantic temperate macroalgae and constitutes their contemporary southern range edge (Lüning, 1990; Provan and Maggs, 2011; Provan, 2013). One of these species is the perennial brown macroalga *Fucus serratus* L. (Coyer et al., 2003). This intertidal, habitat-forming species is confined to the NW corner of the Iberian Peninsula with one range limit in northern Spain and another in northern Portugal (Lüning, 1990; Arribalzaga, 1993). After the recent drastic decline of *Fucus serratus* in N Spain, its presence at its trailing range edge is virtually reduced to scattered populations in large embayments (*rias*) and a few locations semi-exposed to waves in N Spain (Lugo province) and N Portugal (Duarte et al., 2013; Araújo et al., 2014).

In the present work, we aim to characterize inter-population variability in the habitat quality and the performance of peripheral populations of *F. serratus* in two distinct areas in the NW Iberian Peninsula: inside *Rías Bajas* on the western coast and on the northern open coast of Lugo. We also analyzed the local adaptation of the species by using reciprocal transplant experiments. Our purpose is to evaluate the heterogeneity at fine-grained spatial resolution in the range boundary of a species distributions and the implications of this among-population variability in the context of the responses of species to climate change.

2. Materials and methods

2.1. Study area

The surveys and experiments were conducted at four rocky locations in two areas on the Northwestern coast of Spain (Galicia region): the so-called *Rías Bajas*, large embayments on the western coast (hereafter *rias*), and on the open northern coast of Lugo province (Fig. 1). All of the selected locations had abundant populations of *Fucus serratus*: O Freixo (42°47'N, 8°56'W) and Isla de Arosa (42°34'N, 8°51'W) in two different *rias* (Ría de Muros and Ría de Arosa respectively), and San Pedro (43°37'N, 7°19'W) and Peizás (43°35'N, 7°16'W) on the coast of Lugo. Though the Lugo locations are open to the Cantabrian sea, they are partially protected from predominant NW swell as the coastline for both points faces NE (wave roses are available on the web page of Puertos del Estado, <http://www.puertos.es/es-es/oceanografia/Paginas/portus.aspx>). The straight-line distance between the study sites in Lugo and *rias* is approximately 175 km.

2.2. Environmental parameters

To characterize the physical environment at the study sites, data were obtained for four parameters: seawater surface temperature, inorganic nutrient concentrations, salinity and global solar radiation.

Seawater temperature (°C) was measured at each location with Tidbit loggers (Onset Corporation, MA, USA) fixed to the rocky

substratum with stainless steel screws approximately 1.4 m above the Lowest Astronomical Tide, the tidal height where sampling and field experiments were set up (see below). One logger was deployed per location in 2011 (in July at both locations of Lugo and at O Freixo, and since December also at Isla de Arosa). The loggers recorded temperature every 30 min until September 2013. Data were periodically downloaded and two readings were taken per day, coinciding with high tide.

Dissolved inorganic nutrients (nitrate + nitrite and orthophosphate concentrations) were also sampled at the four study locations on three dates (in February, April and June 2013). Three water samples were taken per location and date at rising tide. Samples were placed in 25 ml plastic tubes (seawater previously filtered with polycarbonate filters with 0.2 µm pore size) and frozen within 1 h after collection until they were analyzed in September 2013 using a Skalar auto-analyzer (Skalar, SAN + +).

Surface (0–5 m) salinity data were provided by the Instituto Tecnológico para o Control do Medio Mariño de Galicia (Intecmar, Xunta de Galicia, Spain, www.intecmar.org) from stations close to the studied locations at weekly or biweekly intervals for a two-year period (2012–2013). Monthly average values were calculated. The selected stations were: Vilanova (42°34'N, 8°50'W) close to Isla de Arosa, and O Freixo (42°47'N, 8°56'W) in *rias*, and O Vicedo (43°44'N, 7°41'W) on Lugo coast (Fig. 1). At the latter station, data were only available for 2013.

Global solar radiation (daily average values per month) was provided by Meteogalicia (Xunta de Galicia, Spain, www.meteogalicia.es) from meteorological stations close to the studied locations for the same two-year period (2012–2013). The selected stations were: Lira (42°47'N, 9°7'W), Vilanova de Arosa (42°34'N, 8°48'W) and Corrubedo (42°33'N, 9°1'W) in *rias* and Burela (43°38'N, 7°22'W) and Foz (43°33'N, 7°16'W) in Lugo (Fig. 1).

2.3. Abundance of fucoids and macro-grazers

To determine the biological environment of *F. serratus* populations, the abundance of the target species and other fucoids (*Fucus vesiculosus* L., *Ascophyllum nodosum* (L.) Le Jolis, and *Himanthalia elongata* (L.) S.F Grey, *Bifurcaria bifurcata* R.Ross) and macrograzers was estimated at the four study locations. Sampling was done in the middle of the zone dominated by *F. serratus*, at approximately 1.4 m above the Lowest Astronomical Tide (LAT), during low spring tides in May 2012 for algal cover and in May and July 2012 for grazers. At each location, three sites about 100 m apart were selected and four plots per site (0.25 m² for algae and 0.09 m² for grazers) were randomly chosen. Primary and secondary macroalgae cover (canopy cover) was determined using the point-intercept method with a 50 × 50 cm PVC frame with a grid made of double thread with 81 regularly spaced intersections. Cover was transformed to percentages. A cover of 1% was given to species present in the plots but not recorded. The density of macro-grazers (gastropods and chitons which were identified *in situ*) was estimated using a 30 × 30 cm PVC frame.

2.4. Recruitment of *Fucus serratus*

Natural recruitment of *Fucus serratus* was estimated at all the study locations at mid-shore level (about 1.4 m above LAT) by using artificial substrata. Artificial discs with a rough surface (4 cm in diameter and 5 mm thick) were made using an epoxy resin (Fetadit 55/63; Fetasa, Madrid, Spain), following the methodology of Johnson (1994). Discs were drilled to form a central hole, rinsed in tap water and immersed for 48 h in seawater (previously filtered with Whatman GF/C filters) before use. When reutilized, discs were carefully brushed and submerged in a 10% solution of bleach (sodium hypochlorite) to eliminate any organic remains, and subsequently immersed in tap and seawater, as previously explained. The discs were fixed with stainless steel screws to polycarbonate plates (14 × 14 cm, 3 mm thick) that were then bolted

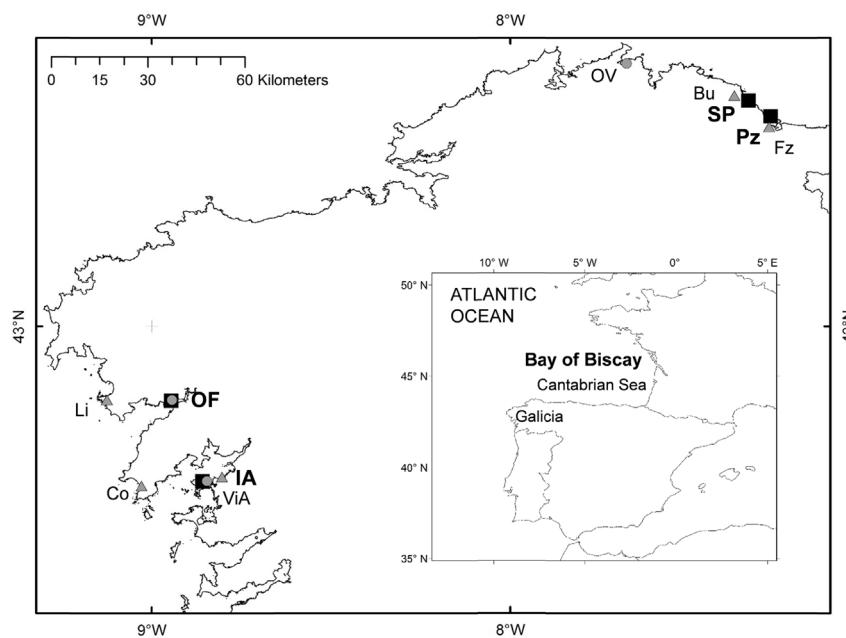


Fig. 1. Study locations in NW Spain (Galicia region). Black squares, locations for surveys and experiments: O Freixo (OF, Ría de Muros) and Isla de Arosa (IA, Ría de Arosa). San Pedro (SP) and Peizás (Pz) in Lugo open coast. Grey circles, coastal stations for seawater salinity data: O Freixo (OF) and Vilanova de Arosa (ViA) in rias, and O Vicedo (OV) in Lugo. Grey triangles, meteorological stations for solar radiation data: Lira (Li), Vilanova de Arosa (ViA) and Corrubedo (Co) in rias, and Burela (Bu) and Foz (Fz) in Lugo.

to rocky platforms at the four study locations. Plates with 2–4 discs each were separated by a minimum distance of 1–3 m under the canopy of *F. serratus*. Discs were left in the field from September 2012 to February 2013 ($n = 24$ per location), and a new trial was carried out from May to October 2013 ($n = 12$ per location). During each trial, a variable number of discs (4–24) was periodically removed, and propagules were counted with the help of a stereo microscope. Specifically, counting was done 1, 2, 4 and 5 months after the start of the assay at each location, except at the locations in Lugo, where the last count was not done in the first period. Germlings of *F. serratus* were counted within hours after collection of the discs from the field. The discs were then placed in aerated seawater and returned to the field the same night or the following day.

2.5. Reciprocal transplant experiment

Transplant experiments were conducted at the study locations in two different periods of the growing season of *F. serratus* (Arrontes, 1993): February to May and June to July 2012 (hereafter named first and second trials). The experiments were set up in the middle of the *F. serratus* belt, at a similar tidal height to that of the recruitment study. We translocated individuals between pairs of locations from the different areas, i.e. rias and Lugo. In the first trial, transplants were conducted between Isla de Arosa (rias) and San Pedro (Lugo) from 10 to 10 February to 5–8 May 2012, and between O Freixo (rias) and Peizás (Lugo) for a shorter period, from 25 to 26 March to 5–8 May (due to logistical constraints). A second trial was carried out between the same locations from 2 to 6 June to 21–23 July. Only non-reproductive, juvenile stages of *F. serratus* (< 14.5 cm in length) were used, and the experiment lasted for short periods of time (2–3 months) to prevent accidental gene flow between distant populations.

We removed 36 of the 54 juveniles selected at each location along with a fragment of the bedrock, mussel shells (*Mytilus galloprovincialis* Lamarck) or barnacles (*Balanus* sp.) where the fronds were attached using a hammer and a chisel and being careful not to damage them. At each location, we transplanted detached individuals back to the same location (procedural controls, hereafter named ‘self-transplants’, $n = 18$) and foreign thalli from another origin (hereafter named ‘transplants’, $n = 18$). Both transplants and self-transplants were numbered with a plastic label and cemented onto the rocky substratum. Unmanipulated juveniles were also labeled and left as controls

($n = 18$). At each location, three groups of 18 algae (6 transplants, 6 self-transplants and 6 controls) were placed in randomly selected sites of about 1 m^2 and separated by 3–5 m. Juveniles used as self-transplants were exposed to similar transportation times and conditions to those of the rest of the transplanted individuals to ensure suitable procedural controls for the transplanting process. At the beginning (mid-February/end of March in the first trial; start of June in the second trial) and at the end of each trial (start of May/end of July, respectively) the length of thalli (from the base of the holdfast to the tip of the longest branch) and the maximum circumference were measured with an accuracy of 0.1 cm. Circumferences < 2 cm were approximated to 2 cm. The volume of individuals was estimated as $v = lc^2$ (l = length, c = circumference; Åberg, 1990). At the end of the experiments, juveniles were gathered, cleaned of epiphytes and dried at 60°C for 48 h. Volume is a good estimation of dry weight for *F. serratus* thalli ($r^2 > 86\text{--}95$ for linear regressions between log dry weight and log lc^2 , see Viejo et al., 2011). Furthermore, total carbon and nitrogen content was determined in a group of dried thalli from the self-transplant and transplant treatments ($n = 3\text{--}7$) in the second trial (i.e. plants collected at the end of July), using a CNH elemental analyzer (Centro de Apoyo Tecnológico, University of Vigo, Spain).

2.6. Feeding preference experiment

As the results of the reciprocal transplant experiments suggested the presence of differential grazing on *F. serratus* fronds depending on the location of origin (see ‘Results’), we set up a preference experiment in the laboratory to test this hypothesis. During the spring tides of May 2013, non-reproductive *F. serratus* fronds were collected at the four study locations and transported to the laboratory under cool and wet conditions. They were left for one night in open PVC tanks with seawater and proper aeration. The feeding assay was initiated the following day at $110\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ of irradiance at the air-water interface and a 13:11 h light:dark photoperiod. Three species of grazers of *F. serratus*, commonly found in the studied area, were collected from the fronds at the Lugo locations and used for the assay: the isopods *Dynamene bidentata* Adams and *Idotea balthica* Pallas, and the gastropod *Gibbula pennanti* Philippi. After the fronds were blotted dry with paper towels and cleaned from all visible epibionts, their wet weights were determined to the nearest 0.1 g. PVC tanks (a total of 26) were filled with filtered seawater (Whatman GF/C filters) and proper aeration.

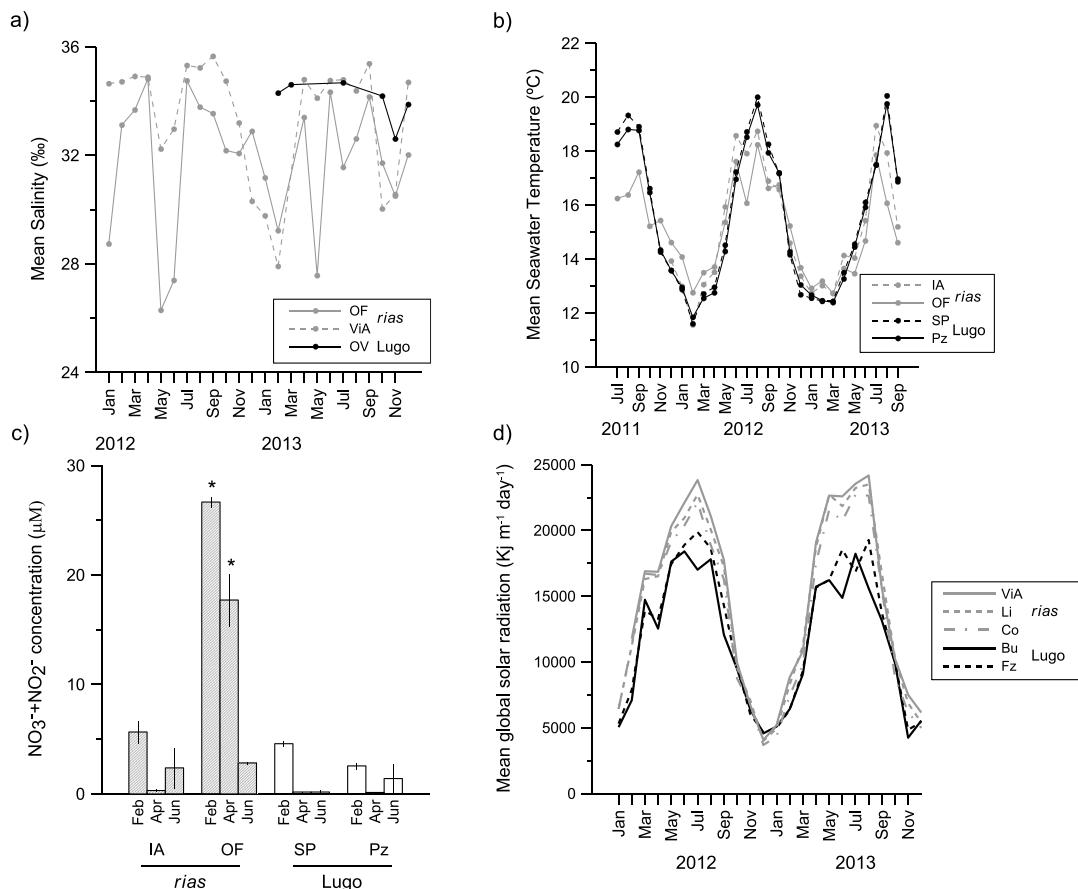


Fig. 2. Values of environmental parameters in *rias* and Lugo coast. (a) Seawater temperature (°C) in the study locations from July 2011 to September 2013. Monthly average values are shown. Location abbreviations as in Fig. 1 (b) Surface salinity (‰) in stations from *rias* and Lugo, over a two-year period (2012–2013) (c) Nitrate + Nitrite concentrations (µM) in study locations (data from 2013). Average values \pm SE are shown, n = 9. Means with asterisks were significantly different from other means within the same Date, based on SNK tests (d) Sunshine hours per month in stations from *rias* and in Lugo coast in 2012–2013.

Temperature was kept at 14.6 (± 0.6) °C (data obtained from two Tidbit loggers placed for 24 h in two tanks, readings taken every 15 min; n = 202), as the average seawater temperature at this time of the year at the target locations in *rias* and Lugo was 14.7 and 14.5 °C, respectively (data from May 2012 and 2013). One frond from each location was placed in each tank. For *D. bidentata* and *G. pennanti* 8 tanks each were used and 6 tanks for *I. balthica* (in all cases 2 grazers per tank). Four additional tanks with algal pieces were left without grazers to control for autogenic changes during the experiment (Renaud et al., 1990). Grazers were not starved prior to the experiment, as starvation may alter feeding behavior (Cronin and Hay, 1996). After one week, algae were inspected for damage, blotted dry and reweighed.

2.7. Statistical analyses

Response variables were analyzed using general linear models (analyses of variance), except the count data of natural recruitment which were evaluated by generalized linear models (GLMs), as this variable had a better fit with a negative binomial than with a Gaussian distribution. Prior to ANOVAs, the assumption of homogeneity of residuals was checked graphically using coplots of standardized residuals versus fitted values (Zuur et al., 2009). In the case of balanced data, Cochran's C-tests (Underwood, 1997) were also used to check this assumption.

Spatial and temporal differences in inorganic nutrients, and differences in nitrogen content of thalli were tested with two-way ANOVAs, with Location and Date, and Location and Treatment as orthogonal and

fixed factors respectively. Differences in the total cover of fucoids and *Fucus serratus* were analyzed through nested ANOVA with Location as a fixed factor (4 levels) and Site as a random factor nested within Location (3 levels). An ANOVA was also used to test for differences in grazer density, adding Date (fixed, 2 levels) as an orthogonal factor in the previous design. When significant differences were found in ANOVAs, *a posteriori* multiple comparisons were carried out using Student-Newman-Keuls (SNK) tests.

The binomial negative instead of the Poisson distribution was used to analyze differences in the recruitment between locations by GLMs, due to the overdispersion of count data (dispersion parameter $\phi > 50$; Zuur et al., 2009). Recruitment analyses were carried out one and four months after the placement of the discs in the field.

Results of the reciprocal transplant experiments were analyzed by two-way ANOVAs, with Location and Treatment as fixed and orthogonal factors, including *a priori* planned comparisons to test for transplant artefacts (Underwood, 1997). The dependent variables used were initial length and volume, elongation (final-initial length) and final dry weights. Since the first trial started at different times at the two pairs of locations, results were analyzed separately. When significant differences were found, the effect of Treatment (control, self-transplant and transplant) either as a main effect or in interaction with Location was split into two *a priori* comparisons: (1) Controls versus Self-transplants (C vs. St) to test for transplant artefacts and (2) Controls (C & St) versus Transplants (T) to test for the effect of the reciprocal transplant. When P-value was < 0.25 in the first comparisons, the effect of transplants was analyzed by comparing St versus T. In this case, the two planned comparisons were non-orthogonal.

For the feeding preference experiment, the amount of each thallus consumed by grazers was estimated as biomass loss (blotted wet mass) and calculated as: $[H_o \times (C_f/C_i)] - H_f$, where H_o and H_f were pre- and post-assay wet weights of each thallus given to the grazers, and C_i and C_f the average pre- and post-experiment wet weights of controls from the corresponding Location of origin. This formula corrects for auto-genic changes during the essay (Cronin and Hay, 1996). Due to methodological problems in weighing the control thalli from San Pedro, this location was excluded from tests. Data were analyzed by a two-way ANOVA with Species (fixed, 3 levels) and Location (fixed, 3 levels) as orthogonal factors. As the consumption of different thalli within the same aquarium is not independent, we only used 1 randomly chosen thallus per aquarium (3 replicates for each combination of Location in the case of *Dynamene* and *Gibbula*, and 2 for *Idotea*). ANOVAs and GLMs were executed using the public domain package R (R Core Team, 2014). Type III Sum of Squares were used for unbalanced ANOVAs (Quinn and Keough, 2002).

3. Results

3.1. Environmental parameters

During submersion/emersion cycles, *Fucus serratus* was exposed to different physical conditions at the two areas: *rias* and Lugo. Sea surface water temperatures (SST) were clearly lower in *rias* than in Lugo during the summer (Fig. 2a; mean SST from July to September over 3 years: 17 °C and 18.59 °C for *rias* and Lugo, respectively). The O Freixo location had the lowest mean SST in the summer months, with differences regarding Lugo locations about 2.4 °C in August 2011 and July 2013, and 3 °C in August 2013. In winter, however, mean temperatures (from January to March) were slightly higher in *rias* (12.94 °C vs. 12.46 °C in Lugo). Variation in SST within years was lower in *rias* than on the Lugo coast. Salinity, on the other hand, presented greater temporal variation in *rias*, with clear reductions in January, February and May (Fig. 2b).

Inorganic nutrient concentrations in the seawater were particularly high at O Freixo in *rias*. This location presented the highest nitrite + nitrate concentrations in February and April (ANOVA, Date × Location interaction effect: $F_{6,24} = 29.20$, $P < 0.001$; Fig. 2c). Orthophosphate values (PO_4^{3-}) were also higher in *rias* locations especially in O Freixo, irrespective of the sampling date (ANOVA for Location effect: $F_{3,24} = 21.31$, $P < 0.001$, SNK test: O Freixo > Isla de Arosa > San Pedro ~ Peizás; non-significant interaction Location x Date, $F_{6,24} = 0.36$, $p = 0.90$). The average values ± SE for orthophosphate concentrations were $0.35 \pm 0.07 \mu\text{M}$ in O Freixo ($n = 9$), 0.22 ± 0.09 in Isla de Arosa ($n = 9$) and 0.12 ± 0.05 in Lugo (pooled locations, $n = 18$).

The *rias* locations were clearly exposed to higher solar global radiation (average daily values per month) than the Lugo locations, particularly in spring-summer (April to September, see Fig. 2d).

3.2. Abundance of fucoids and macrograzers

Total fucoids cover also differed among locations, with Peizás (Lugo coast) presenting the lowest average value (ANOVA, Location effect $F_{3,8} = 8.11$, $p < 0.01$; Fig. 3a). The fucoid canopy was more diverse in the *rias* locations, where patches of *Himanthalia elongata*, *Ascophyllum nodosum* were present along with the co-dominants *Fucus vesiculosus* and *F. serratus* (Fig. 3a), interspersed with spots of grazers (mostly limpets). In contrast, a continuous zone virtually dominated by *F. serratus* was observed at a similar tidal level in Lugo. *F. serratus* abundance was greater in San Pedro (Lugo coast) than at the rest of the locations (ANOVA, Location effect, $F_{3,8} = 9.67$, $p < 0.01$; Fig. 3a, SNK tests $\text{SP} > \text{Pz} \sim \text{IA} \sim \text{OF}$). No significant differences were found in the mean total cover of fucoids or *F. serratus* at small spatial scales (ANOVA, Site effect $F_{8,36} = 1.28$, $p = 0.29$ for fucoid cover, $F_{8,36} = 1.05$, $p = 0.42$ for *F. serratus*).

Macrograzer density was significantly higher in *rias* than in Lugo (ANOVA, significant effect of Location, $F_{3,8} = 15.05$, $p = 0.001$; Fig. 3b). The grazing assemblage was also more diverse at the *rias* locations, where several species of *Littorina* (in O Freixo) or *Patella* (in Isla de Arosa) were abundant in addition to *Gibbula* spp., the only group common to all locations (Fig. 3b). Again, no significant differences of macrograzers abundance were observed at small spatial scales, i.e. differences among sites within locations (ANOVA, Site effect $F_{8,72} = 0.45$, $p = 0.72$).

3.3. Natural recruitment

F. serratus recruitment was fairly consistent in *rias* throughout the year of study (September 2012 to October 2013) in a similar way to Lugo. However, for the period June–September the recruitment in Lugo was significantly lower, particularly at the location of Peizás (Fig. 4a and b), where *Fucus* recruits were only recorded in July 2013.

After one month the average number of germlings settling in San Pedro was higher than at the two *rias* locations in early autumn (from September to October 2012), but very low in spring (May to June) (Fig. 4c; Table 1a). No recruitment was detected in either season in Peizás (Fig. 4c). After four months (September 2012 to January 2013), a general decrease of final recruits remaining on the discs was observed in both study areas, although much more pronounced in Lugo (Fig. 4d; Table 1b). In Peizás no recruits were recorded whereas San Pedro showed a feeble recruitment (and only in September–January period). May–September was the most favorable recruitment period in *rias* (Fig. 4d).

3.4. Reciprocal transplants of juveniles

In the transplant experiments, the initial size of individuals (length and volume) did not differ among locations or treatments (See Table S1 in Electronic Supplementary Material, hereafter ESM, for analyses; mean length ± SE = 9.6 ± 0.1 and $8.9 \pm 0.1 \text{ cm}$ in the first and second trials respectively; mean volume ± SE = 64.7 ± 3.1 and $62.0 \pm 4.8 \text{ cm}^3$, $n = 216$).

In the first trial (February–March to May), no significant differences were found in elongation or final plant weight among treatments or locations (Table 2a,b). Only a marginally significant effect of Treatment ($p \sim 0.05$) was detected for dry weight data in the transplants between Isla de Arosa (*rias*) and San Pedro (Lugo) (Table 2a). Trends of ‘local’ thalli (self-transplants, St) outperforming ‘foreign’ individuals (transplants, T) were only observed for elongation at the Lugo locations (San Pedro and Peizás) and for final dry weights in *rias* i.e. Isla de Arosa and O Freixo (Fig. 5a and b).

In the second trial (June to July), results depended on the recipient location (Table 2c, significant interaction L x Controls [C & St] vs. T). A home advantage, with ‘local’ individuals (C and St) outperforming ‘foreign’ thalli (T), was evident in Isla de Arosa and Peizás (Fig. 5c and d). It is worth noting that the individuals from *rias* which were transplanted into Lugo became severely damaged, presenting breakage (negative elongation) and lower final weights, particularly those from O Freixo transplanted into Peizás (Fig. 5d). In both trials, but especially in the second, thalli transplanted from O Freixo to Peizás showed signs of grazing, and in some cases, only the midrib of the thalli remained (see Fig. S1 in ESM). On the other hand, individuals from San Pedro grew less in this second trial than those from other locations (Fig. 5c and d).

When plants from the same origin were compared, the individuals remaining in *rias* outperformed *rias* individuals transplanted into Lugo. However, self-transplants in Lugo presented an overall similar or even lower growth than the Lugo specimens transplanted into *rias* (Fig. 5; see Table S2 in ESM; significant interactions Location x Treatment).

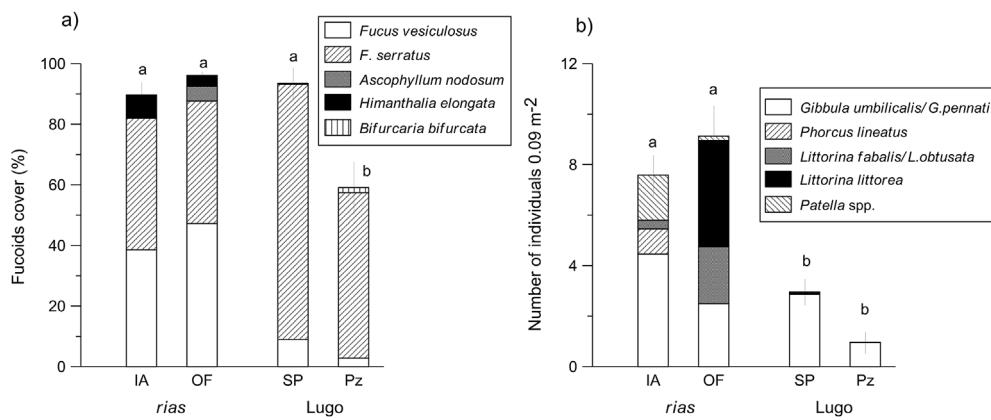


Fig. 3. (a) Percent cover of fucoids in the study locations (IA = Isla de Arosa; OF = O Freixo, SP = San Pedro, Pz = Peizás), data from May 2012, n = 12 (b) Total number of grazers in the same locations, data from May and June 2012, n = 24. Means sharing lower case letters did not differ significantly based on SNK tests.

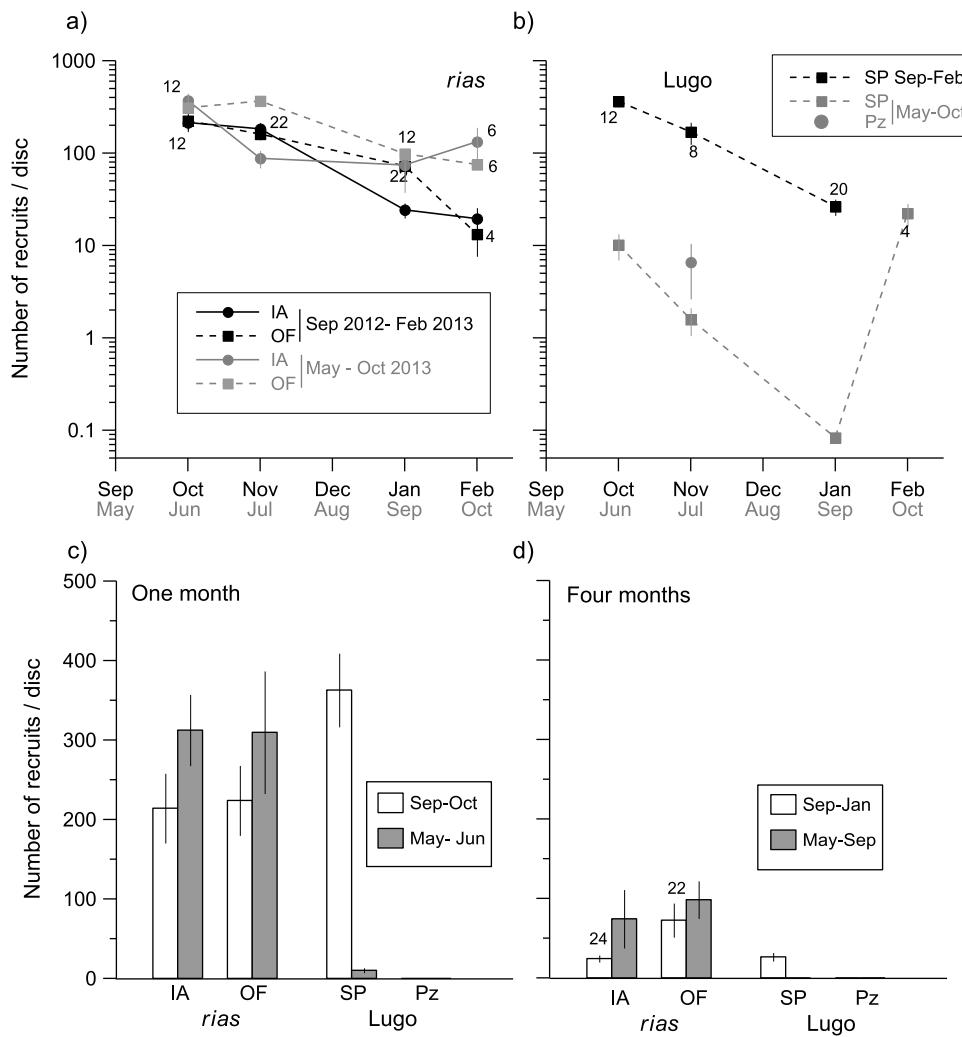


Fig. 4. Natural recruitment of *Fucus serratus* at the study locations. (a–b) Temporal variation in the natural recruitment of *F. serratus* from September 2012 to October 2013 in the locations from *rias* and Lugo respectively (n = 24, except where indicated: number above SE bar). (c–d) One and four-months recruitment (n = 12, except where indicated). Location abbreviations as in Fig. 1.

3.5. Nitrogen content of thalli

The nitrogen content of *F. serratus* juveniles was clearly higher at O Freixo than at the rest of the locations (Fig. 6; ANOVA for Location effect: $F_{3,42} = 47.98$, $P < 0.001$). This is in accordance with the highest nitrate and nitrite seawater concentrations detected at that

location (see Fig. 2c). Individuals from O Freixo also presented a darker color than those from other locations. Nitrogen content in juveniles transplanted from *rias* into Lugo was also higher compared to the fronds remaining in *rias* (juveniles from *rias*, Isla de Arosa and O Freixo: Transplants into Lugo > Self-transplants remaining in *rias*, Fig. 6), while the opposite trend ($S_t < T$) was observed in the thalli from the

Table 1

Negative Binomial Generalized Lineal Model (GLM) testing the effects of Period (P) and Location (L) on the recruitment (germling counts) of *F. serratus* (a) Recruitment after one month in 2 different periods and 3 locations. The interaction P x L was significant and thus parameter estimates of the full model are shown. Isla de Arosa in May–June 2013 was taken as a reference level, and the rest of the estimates are differences from the reference; (b) Four-month recruitment in 3 locations during 1 period. Location abbreviations as in Fig. 1.

a) Periods: Sep-Oct 2012 and May-Jun 2013 (one-month recruitment)				
Locations: IA, OF, SP				
Parameters	Estimate	ES	z Value	p
IA, May-Jun 2013	5.76	0.21	27.44	< 0.001
IA, Sept-Oct 2012	-0.39	0.30	-1.32	0.186
OF, May-Jun 2013	-0.03	0.30	-0.08	0.934
OF, Sept-Oct 2012	0.07	0.42	0.16	0.870
SP, May-Jun 2013	-3.44	0.31	-11.10	< 0.001
SP, Sept-Oct 2012	3.97	0.43	9.24	< 0.001

b) Period: Sep 2012–Jan 2013 (four-months recruitment)				
Locations: IA, OF, SP				
Parameters	Estimate	ES	z Value	p
IA	3.22	0.24	13.33	< 0.001
OF	1.07	0.35	3.08	0.002
SP	0.06	0.42	0.14	0.889

Table 2

ANOVA for the effects of Location and Treatments (C = controls, St = self-transplants T = transplants) on the elongation and final dry weight of *F. serratus* juveniles in the reciprocal transplant experiments. (a) unbalanced data, n = 3–15; (b-c) balanced data, n = 12 and 7 respectively. Location abbreviations as in Fig. 1. In (c) dry weight data were log₁₀ transformed to homogenize variances.

Source	Elongation				Dry weight			
	(a) First trial: IA- SP	df	MS	F	P	MS	F	P
Location, L	1	11.12	1.510	0.226		12.64	3.423	0.071
Treatment, T	2	13.33	1.811	0.175		11.62	3.146	0.053
L x T	2	13.10	1.779	0.181		6.77	1.833	0.172
Residual	45	7.36				3.69		

(b) First trial: OF- -Pz	df	Elongation				Dry weight			
		MS	F	P	MS	F	P		
Location	1	4.25	0.589	0.446	0.06	0.253	0.616		
Treatment	2	7.64	1.058	0.353	0.59	2.337	0.105		
L x T	2	0.36	0.050	0.951	0.54	2.139	0.126		
Residual	66	7.22			0.25				

(c) Second trial: all locations	df	Elongation				Dry weight			
		MS	F	P	MS	F	P		
Location	3	32.06	17.353	< 0.001	1.47	14.131	< 0.001		
Treatment	2	29.90	16.182	< 0.001	0.60	5.799	0.005		
L x T	2								
L x (C vs. St)	3	0.97	0.523	0.668	0.08	0.740	0.531		
L x [(C & St) vs. T]	3	13.44	7.273	< 0.001	0.89	8.538	< 0.001		
Residual	72	1.85			0.10				

Lugo locations. In this case, a higher N content was detected in fronds of Lugo (San Pedro, Peizás) remaining at the same locations in Lugo (Fig. 6; ANOVA, marginally significant interaction Location x Treatment effect: $F_{3,42} = 2.88$, $P = 0.047$).

3.6. Feeding preference experiment

The three species of the tested macrograzers, i.e. the isopods *Dynamene bidentata* and *Idotea balthica*, and the gastropod *Gibbula pennanti*, showed similar feeding preferences (ANOVA, Species effect: $F_{2,9} = 0.60$, $P = 0.571$; Species x Location interaction: $F_{4,9} = 0.61$, $P = 0.665$; Fig. 7). Grazers clearly preferred to eat pieces of *Fucus serratus* from O Freixo than from Isla de Arosa or Peizás (Fig. 7; ANOVA, Location effect: $F_{2,9} = 7.02$, $P = 0.015$; SNK test: Of > IA ~ Pz). A non-significant trend suggested that the thalli from Isla de Arosa grew in the presence of herbivores, especially in presence of *I. balthica* and *G. pennanti* (Fig. 7); negative consumption values indicated a higher final weight in the presence of grazers, after correcting for autogenetic changes in controls, see ‘Material and Methods’).

4. Discussion

Though frequently ignored, populations within the same range boundary may not constitute a homogeneous set. This study revealed environmental heterogeneity and sharp differences in the performance of edge populations of habitat-forming species *Fucus serratus* in the NW Iberian Peninsula. These differences were manifest between locations in *rias* on western shores and on the northern open coast of Lugo province. Both groups of populations have exhibited long temporal persistence and have been present since at least the 1940–1960’s (Fischer-Piette, 1955; Donze, 1968; Pazó and Niell, 1977). However, the detected heterogeneity also suggests divergent prospects for their future viability.

In western *rias*, particularly in the locality of O Freixo, sea surface temperature was cooler in summer, and inorganic nutrient supply was greater than in Lugo. The NW Iberian Peninsula constitutes the northernmost limit of the Canary Upwelling System, one of the major upwelling systems of the world, and therefore highly productive (Kämpf and Chapman, 2016 and references therein). The observed patterns are clearly linked to the presence of intense upwelling events from April to September in *rias* (Fraga, 1981), in contrast to northern coasts of Iberian Peninsula, where upwelling events are weaker and typically limited to the proximity of capes (Botas et al., 1990). As *rias* have independent river basins, the influence of continental runoff from rivers could also contribute to the greater input of inorganic nutrients and the high variability in surface salinity, specifically in the inner part of these large estuaries (Álvarez et al., 2005). Compared to northern open coasts, western *rias* presented greater solar irradiance, higher air temperatures and reduced cloudiness (results of this study, Alcock, 2003, Martínez et al., 2012). Furthermore, locations in *rias* are more sheltered to wave exposure than in Lugo (see Martínez et al., 2012 for a quantification).

In response to spatial heterogeneity in physical conditions, *Fucus serratus* populations from these two areas display a phenotypic differentiation. The geographical distance among peripheral populations of *F. serratus* and the poor dispersal capacity of the species, with embryos recruiting very close to their parents (Arrontes, 1993; Coyer et al., 2003), could enhance genetic divergence. Indeed, evidence indicates that *F. serratus* populations from western *rias* have a distinct genetic pool from populations of N Spain (Ester Serrão’s personal communication). Previous studies have suggested that genetic diversity is reduced within southern-edge populations of *F. serratus* due to isolation and repeated extinctions and recolonizations, limiting its adaptive potential (Coyer et al., 2003; Pearson et al., 2009). However, these events corresponding to expansion and contraction periods of the range boundary did not affect our target populations, which had long temporal persistence, but those situated further east in N Spain which have suffered a drastic reduction in the last decade (nowadays virtually extinct, Viejo et al., 2011; Duarte et al., 2013; Fernández, 2016).

The results of our reciprocal transplant experiments were not consistent across all comparisons, they show a ‘home-site’ advantage with

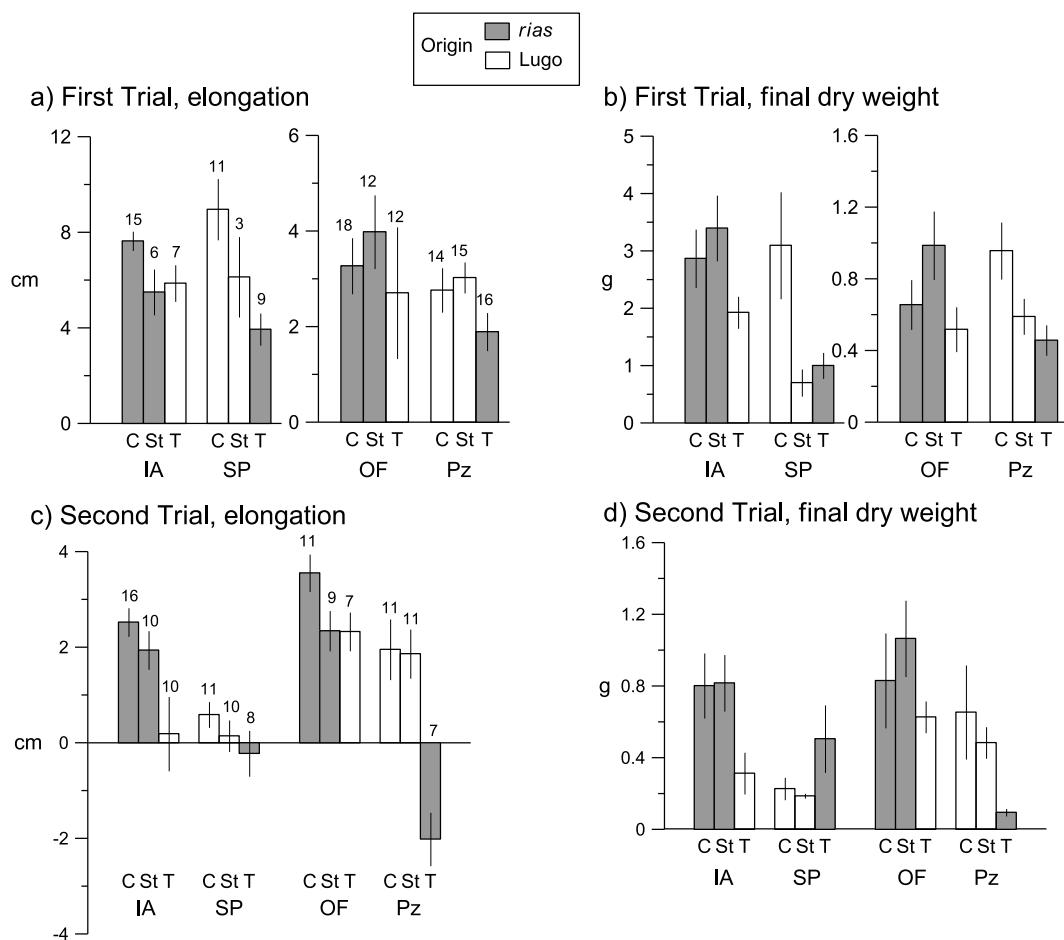


Fig. 5. Reciprocal transplants of juveniles of *Fucus serratus* between rias and Lugo locations. (a,b) First Trial (February–May 2012). (c,d) Second Trial (June–July 2012). Elongation (cm) and final dry weight (g) are shown. Treatment abbreviations: St = self-transplants; C = controls; T = transplants. Data are means \pm SE (the number above SE bar means n replicates). Location abbreviations as in Fig. 1.

higher performance (growth) of local *versus* foreign individuals of *F. serratus*, but only at some locations. Even though juvenile phases were used, carry-over effects of the developmental history in origin locations may also influence these results. To remove environmental effects during development, an additional transplant experiment was carried out with spawned embryos on artificial surfaces in the field. Maternal effects may remain in settled embryos in the field, but a previous study found little support for their influence on the related fucoid *Silvetia compressa* (Hays, 2007). However, our test with embryos failed, due to the presence of new settlement during the experiment, which confounded results. New assays with embryos spawned in the laboratory under controlled conditions should be carried out to test for local adaptation and controlling the potential influence of carry-over effects.

Either adapted or phenotypically influenced by the benign conditions of rias (e.g. cooler seawater and higher nutrient supply), individuals from those large inlets were damaged following their transplantation into Lugo (in ‘home *vs* away’ comparisons *sensu* Kawecki and Ebert, 2004). This was particularly evident in the second trial (June–July). In contrast, some positive growth was detected in the fronds of the reciprocal transplantation from Lugo into rias. This suggests low habitat quality on the Lugo coast, which would present severe physical conditions for these foreign specimens, especially during summer, when differences in water temperature between rias and Lugo were higher, and nutrient availability was reduced.

The contrasting patterns detected in N content of fronds from different origin, support the observed differential growth of transplanted thalli, in particular in the second trial. Hence, the rias fronds transplanted into Lugo which were damaged and did not grow, exhibited

higher N content values compared to fronds remaining in rias, while the opposite trend was observed in thalli from Lugo, where lower N contents were detected in those fronds transplanted into rias. Reduction rates in N macroalgal reserves during the period of active growth are proportional to the growth rates (Rosenberg and Ramus, 1982).

Additionally, foraging could weaken the state of thalli already damaged in a harsh environment. Previous studies have indicated that stressed algae are more susceptible to grazing (Renaud et al., 1990; Cronin and Hay, 1996). In fact, the fronds from O Freixo transplanted into Lugo were the most seriously damaged, often presenting apparent feeding marks (see results and Fig. S1 in ESM), which could be due to differential grazing pressure in foreign *vs.* local fronds. The juveniles grew in O Freixo under high concentrations of inorganic nutrients and correspondingly, exhibited the highest nitrogen contents. Preferential consumption of nitrogen-rich plants may help grazers to obtain sufficient nourishment from low-quality-plant food (Mattson, 1980). Indeed, the results of the laboratory feeding experiment support the preference of Lugo mesograzers for O Freixo’s fronds. The results of this experiment also suggested that fronds from Isla of Arosa grew in presence of herbivores (negative consumption values, see results section). This non-significant trend could be linked to the nutrient enrichment via fecal pellets (e.g. Itoh et al., 2007).

We also detected clear differences in recruitment patterns between areas, i.e. rias *vs.* northern open coast. Recruitment was fairly consistent throughout the whole year of study in both populations in rias, while it was much more spatially and temporally variable in Lugo. These differences were observed despite the exposure of rias populations to a higher density and diversity of grazing gastropods, which scrape on

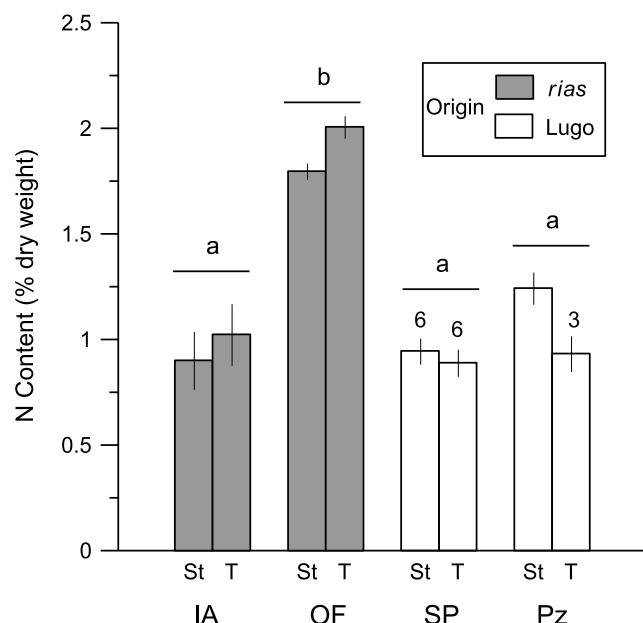


Fig. 6. Nitrogen content (% dry weight) of *Fucus serratus* juveniles of the second trial ($n = 7$ except where indicated, number above SE bar). St = self-transplants, T = transplants. In contrast with Fig. 5, treatments (St, T) are grouped here by the origin location and thus they share the same color (e.g. transplants of Isla de Arosa, IA, are juveniles from Isla de Arosa which were transplanted to San Pedro, Lugo). Means sharing lower case letters do not differ significantly based on SNK tests. Location abbreviations as in Fig. 1.

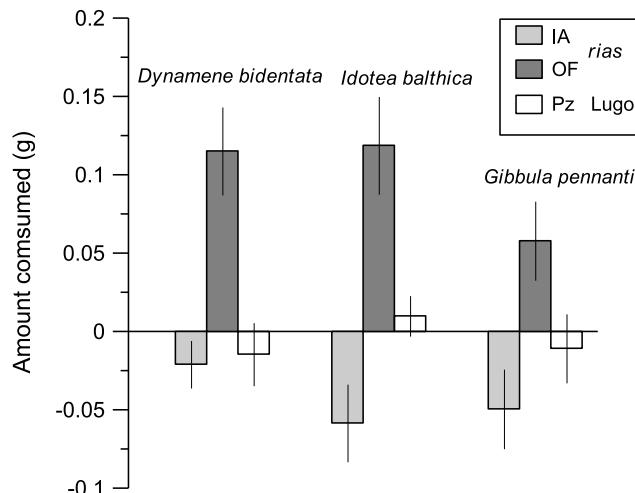


Fig. 7. Change in wet weight (g) of *Fucus serratus* fronds consumed by three grazers in the feeding preference experiment $n = 8$. Location abbreviations as in Fig. 1.

hard substrata to obtain a variety of food sources, including juvenile stages of macroalgae (Johnson et al., 1997). The poor and variable recruitment in Lugo is likely linked to the observed drop in reproduction, as reproductive structures were virtually absent for several months in Lugo populations, while receptacles were detected throughout the year in the *rias* populations (authors pers.obs., see Fig. S1 in ESM). Similarly, Zardi et al. (2015) found that range edge populations of *F. guiryi* in Portugal showed high temporal variability in natural recruitment, given that reproductive fronds were shed during several months.

Changes are undoubtedly occurring in Lugo populations, since two decades ago *F. serratus* was reproductive throughout the whole year in N Spain (Arribalzaga, 1993). Furthermore, in subsequent years from 2014 to 2016, adult plants in Lugo looked seriously damaged, sometimes reduced to the midrib and presenting a crispy aspect (see Figs. S2b-d in

ESM). In our transplant experiment, local fronds from San Pedro hardly grow in the second trial. Thus, nowadays local plants, not just thalli from *rias*, are experiencing increasingly harsh conditions on the Lugo coast, including warming of waters and feasibly reductions of nutrient supply due to increases in summer water stratification (Llope et al., 2006). It seems that the environment is rapidly changing in N Spain, decoupling Lugo populations from these new conditions.

In contrast, the *rias* populations were apparently healthy and reproductive and showed recruitment throughout the year. Western *rias*, which are strongly influenced by upwelling events, feasibly act as contemporary refugia for remnant edge populations of *F. serratus* and other habitat-forming algae of cool-temperate affinities (see also Lourenço et al., 2016). *Himanthalia elongata* is another species still abundant in *rias*, while it recently disappeared from N Spain (Duarte et al., 2013; Fernández, 2016). The refugial effect of *rias* may be promoted by distinct co-varying factors linked to upwelling, as well as by protection from wave action or the influence of river flows. Despite the higher solar radiation measured in these large embayments, cooler water, higher nutrient supply, wave-action protection and increased turbidity and sedimentation due to river inputs may alleviate aerial conditions. A partial muddy cover may protect fronds in these estuarine environments from desiccation and light stresses during low tide (Ladah et al., 2003). Shelter from wave action can avoid or reduce mortality by dislodgement of desiccated specimens after emersion periods (Haring et al., 2002). Furthermore, increases in nutrient supply have been found to prevent or ameliorate frond damage by high light and UV radiation stresses (e.g. Santelices, 1991; Figueroa et al., 2009).

The refugial effect of *rias*, if sustained over time, may mitigate the retreat of *Fucus serratus*' rear-edge predicted by correlative models (see Jueterbock et al., 2013). These models are commonly developed at large spatial scales and therefore may not capture the heterogeneity at fine-grained spatial resolution at the range edge. The prospect of the peripheral populations of this alga in NW Spain and the refugial role of *rias* will strongly depend on climate and upwelling trends, as well as the adaptive potential of these edge populations to face new conditions. However, the future of the coastal upwelling in NW Iberian Peninsula constitutes an unresolved question. Several studies have pointed out the weakness of the upwelling during the last decades (Llope et al., 2006; Pérez et al., 2010; Sydeman et al., 2014). On the other hand, all the recent projections, and particularly those using high-resolution regional models, indicate a strengthening and increase in the frequency of upwelling-favorable winds under future climatic scenarios, both on W and N coasts of the Iberian Peninsula (Casabella et al., 2014; Álvarez et al., 2016; Pires et al., 2016). The projections for this region fit those developed for the rest of major upwelling areas worldwide (Wang et al., 2015). Still, the net result remains uncertain, as there is another factor at play: the warming of coastal waters and the consequent rise in thermal stratification, which could counteract the strengthening of favorable winds (Bakun et al., 2015; Pires et al., 2016). If projections for aerial conditions are also considered, the uncertainty about the future habitat quality for edge populations of *F. serratus* increases. For instance, solar radiation has experienced an upward trend since the early 1980s in the Iberian Peninsula (Sanchez-Lorenzo et al., 2009), but the sign of future changes in this parameter are unclear, and projections vary greatly among climate models (Bartók et al., 2016).

This study highlights the heterogeneity in the environment and the phenotype of populations on the southern, trailing edge of the distribution of habitat-forming alga *F. serratus* and suggests differences in their future viability. To improve the projections of species distributions in future climate change scenarios, further studies should consider this fine-grained spatial variability, focusing on comparisons among peripheral populations within the same range boundary. This is particularly relevant for foundation species, as they constitute fundamental pieces in the functioning of assemblages.

Author contributions

RMV conceived and designed the experiments. LD and RMV conducted fieldwork and experiments. LD analyzed the data. LD and RMV wrote the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.marenvres.2018.02.004>.

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